



NEW DISEASE REPORT

First report of a '*Candidatus Phytoplasma asteris*'-related strain (16Srl-B) associated with three *Sempervivum* cultivars in Hungary

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Plants belonging to the genus *Sempervivum* (houseleek) are succulent perennials forming mats composed of thick, rubbery leaves in rosettes. Several species and varieties of this genus are valued in cultivation as groundcover for dry, sunny locations. In 2018 we observed unusual symptoms on *Sempervivum* growing in commercial nurseries in Hungary. In affected plants a section of the rosette seemed to have emerged upwards above the foliage, the leaves in that part became giant and often also reddish (Figure 1). Between 2019 and 2022 we found these symptoms on five different species or varieties: *Sempervivum* cv. Alpha. Cassiope, Purple Passion and Silberkarneol, and *Sempervivum montanum* grown in commercial nurseries and propagated vegetatively. Approximately 1% of the plants were diseased in the case of the cultivars Alpha, Purple Passion and Silberkarneol (Table 1).

Leaves were collected from five diseased and three symptomless plants of each taxa. Total DNA was extracted by a modified CTAB method (Ahrens & Seemüller, 1992), and nested PCR assays were performed using primer pair fP1/rP7 followed by R16F2n/R16R2 (Lee et al., 1998). Thermocycler conditions consisted of 98°C for 2 minutes, 32 cycles at 98°C for 30 seconds, 55°C or 60°C (fP1/rP7 and R16F2n/R16R2, respectively) for 30 seconds and 72°C for 1 minute, followed by a final extension of 72°C for 10 minutes with KAPA2G

Robust HotStart PCR Kit (Merck KGaA, Germany). Amplicons of the expected sizes (fP1/rP7: 1.8 kb; R16F2n/R16R2: 1.1 kb), were produced from all diseased plants of *Sempervivum* cvs. Alpha, Purple Passion and Silberkarneol, but not from asymptomatic plants of these cultivars. No PCR products were obtained in either PCR round from diseased or asymptomatic plants of *Sempervivum* cv. Cassiope or *Sempervivum montanum*. Amplified fP1/rP7 PCR products were gel purified and ligated into the pJET1.2/blunt cloning vector using a CloneJET PCR cloning kit (Thermo Fisher Scientific, USA). The cloned PCR fragments (at least two from each PCR reaction) were sequenced in both directions by LGC Genomics (Berlin, Germany) using pJET1.2 forward and reverse primers, and the sequence was deposited in GenBank (Accession No. OQ877084). The 16S rRNA gene sequences were identical from all plants of all three varieties and had 99.95% identity with Onion yellows phytoplasma strain OY-M (AP006628) and '*Muscari botryoides*' aster yellows phytoplasma (GenBank ON564434.1) from the '*Candidatus Phytoplasma asteris*' (16Srl-B) subgroup (Figure 2).

We have monitored the evolution of the symptoms for one year and checked the phytoplasma content in three plants of each variety. For all varieties we observed a recovery from the symptoms after three to five months and parallel with that phenomenon, no phytoplasmas were detected by PCR after one year. To our knowledge this is

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FIGURE 1 Symptoms observed in *Sempervivum* cvs. Alpha (a) and Purple Passion (b). Sections of the rosette emerged upwards and the leaves in that section became giant and often reddish. Asymptomatic plant of *Sempervivum* cvs. Purple Passion (c) and Silberkarneol (d).

TABLE 1 The year of testing and the number of plants of each variety assessed for symptoms and the number of symptomatic plants.

Name	Year	Number of diseased plants	Total number of plants assessed visually for symptoms
<i>Sempervivum</i> cv. Alpha	2021	17	1,600
<i>Sempervivum</i> cv. Cassiope	2021	15	1,600
<i>Sempervivum</i> cv. Purple Passion	2020	289	30,000
<i>Sempervivum</i> cv. Silberkarneol	2019	311	30,000
<i>Sempervivum montanum</i>	2021	16	1,800

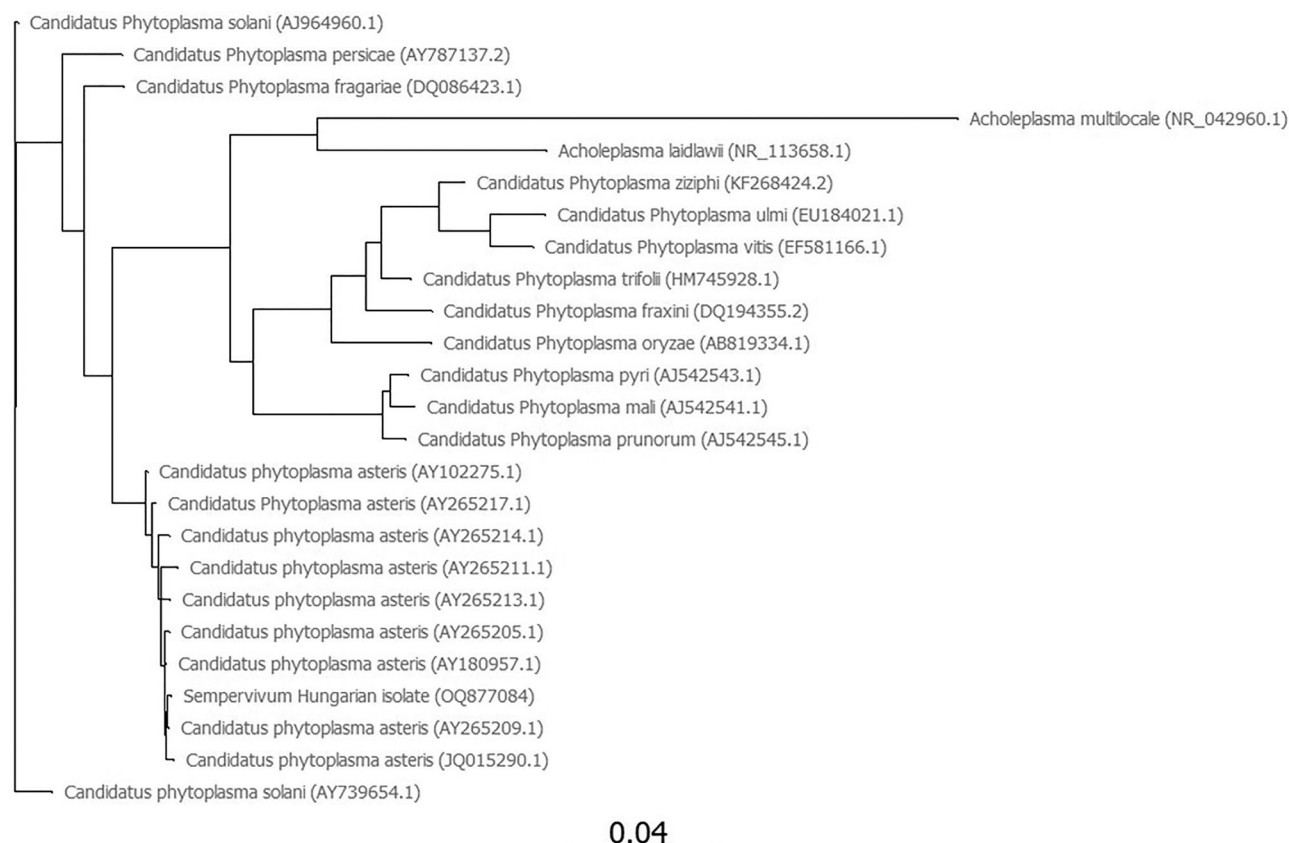


FIGURE 2 Phylogenetic tree constructed by a neighbour-joining method without distance corrections of partial 1.8 kb 16S rRNA gene sequence of the 'Candidatus Phytoplasma asteris'-related strain from this study (OQ877084) and 22 phytoplasmas available in the GenBank database. Two *Acholeplasma laidlawii* sequences were used as an outgroup to root the tree. Bootstrap analyses were done in 1,000 replicates. Phylogenetic analyses were performed using Eugene software (Okonechnikov et al., 2012).

the first identification of a 'Ca. P. asteris'-related strain in *Sempervivum* worldwide.

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